Pathogenetic and physiological mechanisms of poplar ice nucleation active bacterial canker

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Abstract: Using the methods introduced by Bier, X.H., Buchinock, Wang Jing-wen, Shi Rihe *et al.*, different varieties of poplar (poplar-MeixQing, Poplar-A_{100}, Poplar-$Xiaohe_{14}$ *et al.*) were inoculated with poplar ice nucleation active (INA) bacteria respectively in 1997-1999. The water content, relative turgidity, lignin content, phenylalanine ammonialyase (PAL) activity, electrolyte effusion rate, and inorganic element content of poplar bark were measured before and after inoculating. The results showed that after the poplar trees were inoculated with INA bacteria, the moisture content of bark decreased but relative turgidity increased, electrolyte effusion rate increased and had a peak at temperatures of −4 and 5 °C, lignin content increased and positively correlated with poplars' disease-resistance, and the plenylalanine ammonialyase activity increased and also showed a significant positive correlation with poplars' disease-resistance. For the contents of inorganic element, Cu and Fe decreased but K and Zn increased obviously, while Mn, Ca and Mg changed little.

Key words: Poplar; Ice Nucleation Active bacteria; Pathogenesis and physiology
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Introduction

The research results at home and abroad showed that there widely exist some Ice Nucleation Active bacteria (INA bacteria) on the plant (Lindow 1982; Liu 1989; Vail 1971), and it is thought that INA bacteria are one of main factors that cause plant frosted. The research of INA bacteria began with report of Schnellt & Vali. In 1972, they found that rot-leaves were one source of major ice nucleated particle. In 1974, Maki *et al.* separated the *Pseudomonas syringae* from rot leaves of alder, and found these bacteria had high activity of ice nucleation at the temperatures ranged from -2 °C to -5 °C. Since then, more than twenty twenties countries including America, Canada, Japan, Australia etc., carried out some extensive and deep research on population distribution, ecology, ice nucleated mechanism, application about these bacteria. Up to now, INA bacteria have included 31 species and varieties of *Pseudomonas, Erwinia* and *Xanthomonas* (Lindow 1978; Moiier 1987; Zeng 1989). In China, following Xiang Cunti *et al.* who found the Poplar bacterial swollen stem canker caused by *Erwinia* at Zhao Dong City, many Chinese scholars did some researches on cause and state of this disease (Xiang 1992, 2001; Shong 1998; Gao 1996; Dong 2001). They preliminarily defined that the poplar bacterial canker disease resulted from Ice Nucleation Active bacteria (*Erwinia hebicola* (Lohnis) Dye, *Pseudomonas syringae* Sabet *et al.*), probed into the relationship between INA bacteria and poplar bacterial canker, and regarded definitely poplar bacterial canker disease as poplar INA bacterial canker. The study of poplar INA bacterial canker as key subject was listed into the national Eighth Five-Year plan and Ninth Five-Year plan to be researched deeply because that it widely occurred at northeast provinces in China, was extensively epidemic and caused large economical loss. This paper is part of study subject in Ninth Five-Year plan. It further defines the relationship between INA bacteria and poplar INA bacterial canker from the pathogenetic and physiological mechanisms.

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Research methods

Measurement of moisture content and relative turgidity (RT) of bark

The five fresh branches of poplar-MeixQing, Poplar-A_{100}, Poplar-$Xiaohei_{14}$ inoculated with INA bacteria and control

poplar were chosen respectively.

Bier methods (Bier 1964) were used to measure the relative turgidity. Three samples of fresh bark were cut and quickly weighed, then were soaked in distilled water for 24 h till saturated situation. The surface water was absorbed with absorbent paper, then, weighed in saturated situation. After baked dry at the temperature of 95-100 °C, samples were weighed again. The moisture content and relative turgidity (RT) of samples were calculated according to the following formulas.

$$R_T = \frac{w_1 - w_2}{w_3 - w_1} \times 100\ \% \tag{1}$$

$$w_0 = \frac{w_1 - w_2}{w_2} \times 100\ \% \tag{2}$$

Where, R_T is relative turgidity (RT); W_0 is percentage of moisture; w_1 is fresh weight; w_2 is dry weight; w_3 is saturated weight.

Measurement of lignin content of bark

Two-yr-old cutting seedlings of poplar-613 and poplar-A_{100} were soaked in bacterial suspension (5×10^3 single/mL) of INA bacteria (*Pantoea agglomerans*) for 10 h, and moisture was held at the temperature of 25 °C. Control seedlings were treated by sterile water, following by the same steps above.

Referring to the methods introduced by X.H. Buchinock (1981), three 1-g fresh samples were taken and put into test tubes. The compounds of solubility and liposolubility were separated from the samples with acetic acid (10%) and acetone; then hemicellulose and cellulose in the samples were hydrolysed with sulfuric acid (72%). After sediment was washed with distilled water, lignin was oxidized and hydrolysed with potassium dichromate-sulfuric acid solution with concentration of 0.085 mol/L. The surplus potassium dichromate was titrated with standard solution of ferrous sulfate with concentration of 0.5 mol/L and was piloted at the titration end point. According to consumption of standard solution, we can calculate the content of lignin in samples.

Measurement of phenylalanine ammonialyase activity

Two-yr-old cutting seedlings of poplar-613, poplar-A_{100} and poplar-xiaohei$_{14}$ were soaked in bacterial suspension (5×10^3 single/mL) of INA bacteria (*Pantoea agglomerans*) for 10 h, and moisture was held at the temperature of 25 °C. At the same time, the infected branches that were inoculated naturally or artificially were taken.

By methods of Wang Jinweng (1982), three 1-g fresh samples were frosted and fixed at -15 °C, and then the precolding buffer solution of boric acid of 0.2 mol/L (pH8.8, which contained 8 mmol/L mercaptoethanol) was put into mortar to grind, homogenate under ice-bath, and centrifuge under the condition of 4 °C and 10 000 circles per minute for 30 min. Supernatant fluid was used to determine the enzyme activity. The reaction solution of enzyme activity determination, composed of 3-mL boric acid buffer, 1-mL L-phenylalanine (80 mmol/L), and 1-mL enzyme solution, react with supernatant fluid for 60 min at the temperature of 35 °C. Then the reaction was ended by adding 6 mol/L HCl. The various extinction values were measured at the absorbent wavelength of 290 nm. OD value changing 0.01 was regarded as an enzyme activity unit.

Measurement of electrolyte effusion rate of bark

Two-yr-old cutting seedlings of poplar-A_{100}, poplar-613, poplar-MeixQing, poplar-Heixiao$_2$ were soaked in bacterial suspension (5×10^3 single/mL) of INA bacteria for 24 h. The control cutting seedlings were treated by sterile water, following by the same steps above.

(1) Determination of the change of electrolyte effusion rate of bark with time: The treated branches were controlled at the temperature of -10 °C respectively for 0, 5, 10, 15, 25, 35 h, then 0.1-g samples were taken from phloem when the temperature of branches return to natural room temperature. The samples were soaked in non-ion water (26 °C, 120 circles per minute) for 6 h and measured for electric conductivity by DDS-11A. Then the samples were boiled at the temperature of 100 °C and measured for electric conductivity after cooling. At last the electrolyte effusion rate was calculated by the following equation.

$$E = C/B \times 100\% \tag{3}$$

Where: E is electrolyte effusion rate (%), C is control electric conductivity, and B is boiled electric conductivity.

(2) Determination of the change of electrolyte effusion rate of bark with temperature: The method of sampling was the same as above. 0.1-g samples were put into test tubes and treated at the temperatures of 24 °C, 10 °C, 5 °C, -4 °C, -8 °C, -12 °C for 60 min, repeated three times (5 samples each repeat). Then test tubes were taken out and added non-ion water of 10 mL. The tubes were covered with plug and controlled at the temperature of 26 °C for 6 h. The methods of measurement and calculation were the same as above.

Measurement of inorganic element of poplar

Three 0.3-g samples taken from 5 fresh branches of infected poplar-Beijing605 and poplar control were put into 500-mL beaker, added 6-mL nitric acid and 1-mL perchloric acid, then covered with watch glass evaporate and heated on the hot electric plate of 500 watts (Shi 1989). After then, by a ratio of 1:1, 1.5-mL hydrochloric acid was added to solve the samples. The samples were transferred into colorimeter tubes of 25 mL, fixing volume with deionized water. This solution was used to measure content of Cu, Zn, Mn, and Fe. When measuring the content of K, Ca, and Mg, 1-mL sample was taken out from 25-mL colorimeter tube, and put into another 25-mL colorimeter tube, with adding 0.5-mL NaCl and SeCl (10%) for fixing volume.

Results and analysis

Moisture content and relative turgidity (RT) of bark

The moisture content of bark decreased but relative turgidity (RT) increased after poplar INA bacterial canker occurred (Table 1), which indicated that poplar was apt to be infected when the relative turgidity increased. For example, poplar-A_{100} and poplar-MeixQing are easier to be infected than poplar-Xiaohei$_{14}$ because they have higher RT, which accords with practical situation of investigation.

Lignin content of bark

The lignin contents of both inoculated poplar-A_{100} and poplar-613and control ones increased after held moisture and tended to be stable at certain time, but the accumulated speed of lignin for inoculated poplars is much higher than that of control. It showed that susceptible variety was much sensitive to invasion of INA bacteria (Table 2).

Table 1. Moisture content and relative turgidity (RT) of various varieties of poplars

Item	Mei×Qing infected	Control	A_{100} infected	Control	Xiaohei$_{14}$ infected	Control
Percentage of moisture	110.56	110.78	111.81	112.98	120.46	133.66
Relative turgidity (RT)	68.03	63.83	62.54	53.95	31.34	32.64

Table 2. Variety of lignin content of poplar-A_{100} and poplar-613

Varieties	Disposal	Lignin content in different moisture-holding days					
		0d	1d	2d	3d	4d	5d
Poplar- A_{100}	Inoculated	2.76	4.12	4.81	4.95	5.56	5.65
Poplar- A_{100}	Control	2.76	3.54	3.86	3.91	4.78	4.87
Poplar -613	Inoculated	3.25	4.00	4.06	4.46	4.49	4.55
Poplar- 613	Control	3.25	3.86	3.91	4.09	4.32	4.43

Phenylalanine ammonialyase activity variety

The various varieties of poplar inoculated with INA bacteria all showed an increase in the phenylalanine ammonialyase activity, of which resistant poplar-613 had higher phenylalanine ammonialyase activity than that of poplar-Xiaohei$_{14}$ before and after inoculation (Table 3).

Table 3. Phenylalanine ammonialyase activity change of various varieties after disease occurring

Varieties	Extinction value in moisture-holding days			Infection
	0d	1 d	2 d	
Poplar-613	0.701	0.702	0.770	0.814
Poplar-A_{100}	0.670	0.731	0.792	0.895
Poplar-Xiaohei$_{14}$	0.250	0.430	0.454	0.468

Electrolyte effusion rate of bark

The electrolyte effusion rates changed with the time. It decreased at first, then gradually increased, and tended to be stable when reached a certain value (Table 4).

Electrolyte effusion rate of bark changing with temperature

The electrolyte effusion rate of bark of the poplar inoculated with INA bacteria was higher than that control. INA bacteria are most active and the electrolyte effusion rate is highest at temperature of –4 and 5 °C and lowest at the temperature of -12 °C and 24 °C. The electrolyte effusion rates of infected poplar-A_{100} and poplar-MeixQing are higher than that of resistant poplar-613 (Table 5).

Inorganic element of infected host

The content of Cu and Fe decreased obviously after host infected, that of K and Zn increased obviously, and the content of Mn, Ca and Mg changed little (Table 6).

Table 4. Electrolyte effusion rate of bark changing with time at low temperature

Varieties	Effusion rate					
	0 h	5 h	10 h	15 h	25 h	35 h
Poplar-A_{100} inoculated	24.118	17.391	27.073	35.167	24.560	22.298
Poplar-A_{100} control	31.304	17.00	26.639	31.317	25.938	25.225
Poplar- 613 inoculated	17.500	13.197	27.073	45.137	20.558	50.329
Poplar -613 control	23.125	16.244	26.639	36.592	23.706	41.250
Poplar-Heixiao$_2$ inoculated	35.00	22.879	24.736	25.941	45.444	68.803
Poplar-Heixiao$_2$ control	20.00	18.519	50.515	53.081	46.266	50.323
Poplar Mei×qing inoculated	30.185	15.914	23.719	37.817	55.690	61.051
Poplar-Mei×qing control	34.483	13.25	24.688	49.202	51.308	68.252

Table 5. Electrolyte effusion rates of different varieties of poplar at different temperatures

Varieties	Effusion rate					
	-12°C	-8°C	-4°C	5°C	10°C	24°C
Poplar-A_{100} inoculated	38.89	49.20	49.80	56.00	55.77	45.22
Poplar-A_{100} control	25.27	26.32	36.87	43.43	32.91	38.54
Poplar-613 inoculated	21.60	22.58	22.87	35.48	18.31	16.69
Poplar-613 control	21.04	21.05	25.70	28.65	24.58	25.75
Poplar-$Heixiao_2$ inoculated	32.48	38.52	42.06	49.74	52.17	40.80
Poplar-$Heixiao_2$ control	44.34	45.00	45.68	45.53	48.99	41.94
Poplar-Mei×qing inoculated	54.95	56.76	62.96	57.73	57.21	54.65
Poplar-Mei×qing control	49.82	54.27	57.18	42.74	47.42	45.92

Table 6. Content of inorganic element after poplar-BeiJing605 infected

Varieties	Element						
	Mg	Cu	Zn	Mn	Fe	K	Ca
Poplar-Beijing605 infected	0.451	0.059	1.300	0.230	0.816	1.602	5.545
Poplar-Beijing605 control	0.496	0.129	1.067	0.274	2.400	1.224	5.555

Conclusions

After INA bacteria canker occurred, the water content decreased and relative turgidity (RT) rose in bark. It shows that the poplar is apt to be infected when relative turgidity rise. The lignin content has positive correlation with resistance of poplar varieties. The varieties of poplar infected are more sensitive to invasion of INA bacteria. The speed of lignin content accumulation of poplar inoculated with INA bacteria is higher than that of control. After inoculated with INA bacteria, all varieties of poplar increased in the phenylalanine ammonialyase activity. The phenylalanine ammonialyase activity of resistant poplar-613 is higher than that of poplar-$Xiaohei_{14}$ before and after inoculated. The electrolyte effusion rate of poplar bark after inoculated with INA bacteria was higher than that of control. The INA bacteria are most active at the temperature of -4 and 5 °C. The electrolyte effusion rates of susceptible poplar-A_{100} and poplar-MeixQing were higher than that of resistant poplar-613. With the time longing of treatment at low temperature, the electrolyte effusion rate firstly decreased; then gradually rose; at last, it tended to be stable at certain value.

After the host was infected, the content of Cu and Fe decreased obviously, that of K and Zn increases obviously and the content of Mn, Ca and Mg doesn't change obviously.

References

Bier, J.E. 1964. The relation of some bark factors to canker-susceptibility [J]. Phytopathology., **54**: 250-253.

Buchinock, X.H. [Russia]. (Jin Jiahai translated). 1981. Plant biochemistry analysis methods [M]. Beijing: Scientific Press, p50-78.

Dong Airong, Xiang Cunti, Liu Xuefeng *et al.* 2001. Regional epidemic laws of poplar ice nucleation active bacterial canker [J]. Journal of Forestry Research, **12**(3): 165-168.

Gao Guoping. 1996. Screening test of main poplar variety resisting bacterial canker at Liaoning Province [J]. Forest Research. (special publications) **9**: 149-153.

Lindow, S.E. Hirano, S.S. and Barchet, W.R. 1982. Relationship between ice nucleation frequency of bacteria and frost injury [J]. Plant Physiol, **70**(4): 1090-1093.

Lindow, S.E., Arny, D.C., Upper and C.D. 1978 Erwinia herbicola: A bacterial ice nucleus active in increasing frost injury to corn [J]. Phytopathology, **63**(3): 523-527.

Liu Jianhua *et al.* 1989. The research survey of INA bacteria and plant frost [J]. Plant Protection, (4): 41-43.

Moller, W.J. Reil, W.Q. *et al.* 1987. Fireblight forecasting and control incalifornia. Anhistorical perspective [C]. In Proc. Forth Int. Conf. Plant path. Bact. Angers, pp. 457-459.

Shong Fuqiang, Gao Changqi. He Pingxun *et al.* 1998. Research of essential factor of poplar INA bacteria canker occurring [J]. Forest Pest and Disease, (4): 5-6.

Shi Rihe. 1989. Plant nutrition principle [M]. Jiangsu: Scientific Press of Jiangsu Province, 30-45.

Vali G.. 1971. Quantitative evaluation of experimental results on the heteragenous freezing nucleation of supercooled liquids [J]. J. Atomos. Sci., **28**: 402-409.

Wang Jingwen *et al.* 1982. Research of plant phenylalanine ammonialyase. The action of phenylalanine ammonialyase at the course of resisting murrain [J]. Journal of Plant Physiology, **8**(1): 35-43.

Xiang Cundi, Shao Liping compiled. 1992. Poplar disease integrate control technology (No.1volume) [M]. Harbin: Northeast Forestry University Press, 119-167.

Xiang Cundi, Dong Airong, Liu Xuefeng *et al.* 2001. Key factors for causing poplar ice nucleation active bacterial canker and its control techniques [J]. Journal of Forestry Research, **12**(3): 157-164.

Zeng Dapeng, Zhang Yongxiang, Chao Longjun *et al.* 1989. Research of INA bacteria in poplar of North. Plant Protection [J]. (4): 41-43.